Rapid Translation System
RTS pIVEX MBP Fusion Vector

Cat. No. 3 268 985

Version 1, December 2001
Store at −15 to −25°C

1. Preface

Kit contents

Vial	Label	Contents and use
1	pIVEX-MBP	• 10 µg (20 µl) plasmid • cloning vector with cleavable N-terminal MBP fusion partner • additional N-terminal His-Tag for detection and purification • contains a multiple cloning site (MCS)

Safety Information

None of the bottles contain hazardous substances in reportable quantities. The usual precautions taken when handling chemicals should be observed. Used reagent can be disposed off in waste water in accordance with local regulations. In case of eye contact flush eyes with water. In case of skin contact wash off with water. In case of ingestion seek medical advice.

Stability of pIVEX vectors

Vectors are stable for 1 week at 2–8°C and for 2 years at −15° to −25°C. Repeated freezing and thawing decreases the amount of supercoiled plasmid.

2. Introduction

Roche's Rapid Translation System RTS pIVEX Vectors are designed for high-level expression of proteins in the cell free RTS *E. coli* system. The vectors contain all regulatory elements necessary for *in vitro* expression based on a combination of T7 RNA polymerase and prokaryotic cell lysates. Cloning into pIVEX Vectors (plasmid for In Vitro EXpression) allows optimal protein expression in all RTS *E. coli* systems (see 4.7 Related products). The vector pIVEX-MBP allows the expression of proteins fused to the C-terminus of MBP (maltose-binding protein). This generally leads to enhanced solubility of the desired protein. An additional His_6-tag at the N-terminus of the fusion protein provides the possibility to detect and purify proteins of interest. MBP can be cleaved off using Factor Xa restriction protease.

3. Cloning into pIVEX vectors

3.1 Vector description

Vector nomenclature

The general architecture of the MBP fusion vector is shown in Fig. 1. The target gene cloned into the multiple cloning site will be expressed as MBP fusion protein with a hexa-histidine tag at its N-terminus (the MBP fusion partner including Factor Xa recognition site has a molecular weight of 45.5 kDa).

Both the MBP fusion partner and the hexa-histidine (His_6) -tag allow easy detection and purification of the expressed protein. For purification protocols please refer to our website www.proteinexpression.com.

For a detailed vector map refer to chapter 4.3.

Functional elements of pIVEX MBP

Fig. 1: Functional elements of pIVEX-MBP.

Abbreviations
T7 P = T7 Promoter, RBS = Ribosomal binding site, MBP = maltose-binding protein, Xa = Factor Xa restriction protease cleavage site, MCS = Multiple cloning site for the insertion of the target gene, T7 T = T7 Terminator

The complete vector sequence can be viewed and downloaded from the Roche Applied Science protein expression web site. For additional vectors with alternative tags please refer to our current catalog or to our websites **http://www.roche-applied-science.com** and http://www.proteinexpression.com.

3.2 Selecting the cloning strategy

The plasmid pIVEX-MBP as expression vector provides the possibility to select between two cloning strategies depending on your demands:

- The *Nco I/Sma* I restriction site combination for cloning provides optimal flexibility to switch into all available pIVEX vectors and generally results in good expression efficiencies. Stop codons in all three reading frames are located downstream of the MCS to allow switching between vectors providing N- and C-terminal tags. Once a DNA fragment is prepared (e.g. by PCR), cloning into different pIVEX vectors can easily be done in parallel or successively.
- Blunt-end cloning into the *Stu* I site allows the expression of a protein that - after Factor Xa cleavage - contains no additional amino acids derived from the vector. For directional cloning we recommend to use the *Stu* I and *Xma* I (= *Xma* CI) sites of pIVEX-MBP. The PCR product to be cloned has to begin with a blunt end (directly with your specific sequence) and must end with a stop codon followed by a restriction site and 6 to 10 additional bases to allow complete digestion. If your target gene has an internal *Xma* I site, take an enzyme producing compatible ends (e.g. *Pin* AI) or another restriction enzyme cutting in the MCS of pIVEX-MBP. To avoid blunt end cloning, do not use *Sma* I as the ligation will not be directional in this case. For an example, see section 4.1.2.

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3.2 Selecting th cloning strategy, continued

If you want to take advantage of the flexibility of our vector sets, select the cloning strategy strictly according to the following decision matrix depending on the existence of internal restriction sites in your target gene.

IF...	THEN...
The target gene is free of internal *Nco* I and *Sma* I sites	• **Use *Nco* I and *Sma* I sites**. ***Note***: The second amino acid will be changed in most cases. Design primers according to the example in chapter 4.1.1.
The target gene has an internal *Sma* I site (generates blunt ends)	• **Use an alternative blunt end restriction site** in the reverse primer that does not cut inside of your target gene (e.g. *Eco* RV, *Ssp* I, *Sca* I). • Cut pIVEX-MBP with *Nco* I and *Sma* I.
You want to avoid blunt end cloning at the 3' end	• **Use *Xma* I**, if your gene does not contain an internal *Xma* I site. *Xma* I recognizes the same sequence as *Sma* I but leaves a cohesive (sticky) end. Alternatively, you can use *Pin* AI, *Sgr* AI, *Bse* AI, or *Ngo* MIV which generate compatible, cohesive (sticky) ends. • Cut pIVEX-MBP with *Nco* I and *Sma* I.
The target gene has an internal *Nco* I site	• **Use a *Rca* I or *Bsp* LU11 I site** in the forward primer, if no *Rca* I or *Bsp* LU11 I site is present in the target gene. These enzymes generate cohesive (sticky) ends compatible with *Nco* I. • Cut pIVEX-MBP with *Nco* I and *Sma* I.
The target gene has internal *Nco* I, *Rca* I and *Bsp* LU11 I sites	• **Introduce a *Nde* I** sequence into the forward primer. • Use the *Nde* I site in pIVEX-MBP.
The target gene has internal *Nco* I, *Rca* I, *Bsp* LU11 I and *Nde* I sites	• Check for any of the additional restriction sites present in the MCS of pIVEX-MBP. • Include one of these sites into the forward primer **or** • Eliminate the restriction site by mutation (e.g. conservative codon exchange, refer to the literature given at the end of chapter 4.1) **or** • Prepare a cloning fragment by limited digestion if desired restriction site is present in the gene (refer to the literature given at the end of chapter 4.1).

3.3 Cloning procedure

3.3.1 Primer design for PCR cloning

Rules for primer pair design

- Use forward and reverse primers consisting of about 20 bases complementary to the gene, the restriction sites of choice (in frame), and 5-6 additional base pairs to allow proper restriction enzyme cleavage (for examples see chapter 4.1.1).
- Using a 5'-phosphorylated sense oligo for cloning in *Stu* I provides the possiblitiy to use a dephosphorylated vector to reduce background caused by religation.
- For efficient digestion with *Nde* I or *Not* I the number of additional basepairs must be higher. Include 8 additional basepairs in the primer to cut your PCR product with *Nde* I and 10 additional basepairs to cut it with *Not* I.
- Design forward and reverse primers with comparable (±2°C) melting temperature (for calculation of melting temperatures see appendix 4.1.1).
- Try to minimize secondary structure and dimer formation by means of primer design.
- High quality primers (purified on HPLC or acrylamide gels) are recommended.

3.3.2 Restriction digest of the pIVEX vectors

Digestion of pIVEX vectors for cloning

Briefly centrifuge down the contents of the vial with the pIVEX vector.

- Digest pIVEX vector using the appropriate restriction enzymes and buffers (for restriction enzymes and buffers please refer to our current catalog).
- Run an agarose gel to control the reaction and to separate the linearized vector from undigested vector and smaller fragments.
- Isolate and purify the fragment with the correct size from the gel (e.g. using the Agarose Gel DNA Extraction Kit).

Examples:

Digestion with...	Procedure
Nco I and *Sma* I (or *Xma* I)	• Digest 2 µg (4 µl) of DNA with 20 units of *Sma* I in 20 µl of 1× buffer A at 25°C (or 20 units of *Xma* I in buffer M at 37°C) for one hour. • Check an aliquot to be sure that the plasmid is linearized. • Add 20 units of *Nco* I and digest for another hour at 37°C.
Nde I and *Sma* I (or *Xma* I)	• Digest 2 µg (4 µl) of DNA with 20 units of *Sma* I in 20 µl of 1× buffer A at 25°C (or 20 units of *Xma* I in buffer M at 37°C) for one hour. • Check an aliquot to be sure that the plasmid is linearized. • Add 20 units of *Nde* I and digest for another hour at 37°C. (see 4.1.3 for additional hints concerning *Nde* I digests)
Stu I and *Xma* I	• Digest 2 µg (4 µl) of DNA with 20 units of *Xma* I and 20 units of *Stu* I in 40 µl of 1× buffer A at 37°C for one hour. • Optional: Digest the vector successively and check an aliquot after the first reaction.

Phosphatase treatment of the digested pIVEX vectors

This step is optional in the case of cohesive end cloning but necessary for ligation of blunt ended inserts. ***Note:*** Do not dephosphorylate the vector if you want to clone your gene with *Stu* I unless you used a 5'-phosphorylated sense primer or the PCR fragment has to be phosphorylated with a kinase.

- Treat 300 ng of digested pIVEX vector with 3 units of shrimp alkaline phosphatase in a total volume of 10 µl in 1× phosphatase buffer for 90 min at 37°C.
- Inactivate the shrimp phosphatase by heating to 65°C for 15 min.

3.3.3 Preparation of the inserts

Generation of PCR fragments

- Primer design
  Design PCR primers according to section 3.2.
- PCR conditions
  Optimal reaction conditions depend on the template/primer pairs and have to be calculated accordingly.
  - To avoid nonspecific products and misincorporation, try to keep cycle number as low as possible (<25).
  - To reduce the error rates use a high fidelity PCR system that includes a proof-reading enzyme (e.g. Expand High Fidelity PCR-System), especially with templates longer than 2 kb.
- Restriction digest
  Digest the PCR product using the restriction sites introduced with the primers.
  Note: The cutting efficiency of many restriction enzymes is reduced if their recognition sites are located less than 6 base pairs (for *Nde* I 8 basepairs and for *Not* I 10 basepairs) from the 5' end. Therefore, restriction digests require higher enzyme concentrations and longer incubation times (see 4.1.3 for additional hints concerning *Nde* I and *Not* I digests).
- Purification of the PCR fragment
  Run the digested PCR product on an agarose gel. Excise the fragment with the correct size from the gel and purify it (e.g using the Agarose Gel DNA Extraction Kit).

Subcloning of PCR fragments using PCR cloning vectors

Restriction enzymes often do not cut efficiently if the restriction site is located at the very end of a fragment. The completeness of the digest is difficult to analyze due to the small difference in size. Subcloning of PCR fragments using PCR cloning vectors can circumvent this step of uncertainty. An instruction for this strategy is given in the appendix (4.1.4).

Excision of restriction fragments from existing vectors

Under certain conditions the target gene can be excised from an existing vector construct. This strategy can be applied if the gene is already flanked by restriction sites contained in the MCS of the pIVEX vector (see chapter 4.3 for a vector map). **In any case, check whether the start codon AUG and the stop codon are in the correct reading frame.**

3.3.4 Vector ligation, transformation, and purification

Ligation
Ligate the purified DNA fragment into the linearized pIVEX vector (using e.g. the Rapid DNA Ligation Kit). For ligation of DNA fragments digested with *Nde* I see chapter 4.1.3.

Transformation
Transform a suitable *E. coli* strain (e.g. XL1 blue) to amplify the expression plasmid.

Amplification of the plasmid in *E. coli*
Prepare a suitable amount of plasmid for the subsequent transcription-translation reactions. For a single 50 µl reaction, 0.5 µg plasmid are required. For a single 1 ml reaction 10–15 µg plasmid are required. Preparation of a sufficient amount of plasmid for multiple reactions including characterization by sequencing (see 3.3.5) is recommended. Geno Pure Plasmid Midi or Maxi Kits are best suited for this purpose (see 4.7 Related products).

Purity of the plasmid preparation
The purity of plasmids obtained from commercially available DNA preparation kits is sufficient for the use as template in the Rapid Translation System. When DNA purity is insufficient ($OD_{260/280} \leq 1.7$), a phenol treatment to remove proteins (e.g. traces of RNase) may enhance expression. Do not use smal scale (Mini) DNA preparation methods.

3.3.5 Analysis of the new expression vector

Restriction mapping
Successful cloning should be verified by restriction mapping of the construct and subsequent analysis on an agarose gel. We recommend using a restriction enzyme with a single cleavage site in the vector (like *Cla* I or *Bam* HI) together with another enzyme that has one or two cleavage site(s) within the target gene.

Sequencing
The generated expression vectors should be sequenced to verify the correctness of the PCR generated DNA fragments and correct cloning. Use a 5' primer and a 3' primer binding upstream of the MCS and of the T7 terminator sequence.

- Recommended 5'- primer:
  5'- GCAGCTCGAACAACAACAAC -3'
- Recommended 3'- primer:
  5'- GCTAGTTATTGCTCAGCGG -3'

4. Appendix

4.1 Additional information for cloning

4.1.1 Example for designing a *Nco* I/*Sma* I primer pair

Target gene sequence (example):

```
   Met
5'-ATGCTAGCAAACTTACCTAAGGGTNNN  Stop
       NNNTTGTTCCCGTTCAAAATATTGTAA-3'
3'-TACGATCGTTTGAATGGATTCCCANNN
       NNNAACAAGGGCAAGTTTTATAACATT-5'
```

For cloning a gene into a pIVEX vector use:

- a forward primer with *Nco* I site (bold letters):

```
5'-XX XXX XCC ATG GTA GCA AAC TTA...
                  ...CCT AAG GGT-3'
```

$T_m = 12 \times 2°C + 8 \times 4°C = 56°C$

Note: The second amino acid will be mutated in this example. This is true for all cases (ca. 75%) where the target sequence has A or C or T (not G) after the ATG start codon and a G is required in the primer sequence to introduce the *Nco* I site. If you resign the possiblity to recut the inserted DNA with *Nco* I, you can use e.g. *Rca* I or *Bsp* LU11 I that generate ends compatible with *Nco* I, but have an A and a T in the sixth position of the recognition sequence, respectively.

- and a reverse primer with *Sma* I site (bold letters):

```
5'-XXX XXX CCC GGG CAA TAT TTT GAA CGG...
                              ...GAA CAA-3'
```

$T_m = 14 \times 2°C + 7 \times 4°C = 56°C$

Formula for melting point (T_m) calculation
T_m = (number of A+T) × 2°C + (number of G+C) × 4°C
Optimal annealing temperatures for PCR are 5 to 10°C lower than the T_m values of the primers.

4.1.2 Expression of proteins without additional vector derived amino acids

If you want to express a protein without any additional amino acids, we recommend two strategies:

- Blunt-end cloning into the *Stu* I site of pIVEX-MBP will result in a protein that contains no vector derived amino acids after cleavage with Factor Xa provided that the PCR fragment starts with the specific gene sequence and includes a stop codon. Using a 5'-phosphorylated sense oligo provides the possibility to use a dephosphorylated vector and therefore to reduce cloning background caused by religation.

Example:

```
  Met
5'-ATGCTAGCAAACTTACCTAAGGGTNNN  Stop
     NNNTTGTTCCCGTTCAAAATATTGTAA-3'
3'-TACGATCGTTTGAATGGATTCCCANNN
       NNNAACAAGGGCAAGTTTTATAACATT-5'
```

Forward primer (without restriction recognition site)

```
5'-(P)-ATG CTA GCA AAC TTA CCT AAG-3'
```

Reverse primer (with *Xma* I recognition sequence in italics)

```
5'-XXX XXX CCC GGG TTA CAA TAT TTT GAA...
...CGG GAA-3'
```

- As a second strategy for expressing a protein without any additional amino acids or in the case of an internal Factor Xa cleavage site present in your protein, we recommend to insert an additional protease cleavage site (e.g. for enterokinase) directly upstream of your target gene sequence into the forward primer. In this case, use a restriction enzyme (e.g. Nco I) producing cohesive ends for easier cloning.

Example:

```
                NcoI
5'-XX XXX XCC ATG...
...GTA GAT GAC GAC GAC AAG NNN NNN...-3'
      Asp-Asp-Asp-Asp-Lys-target gene
                      ↑
                enterokinase cleavage site
```

4.1.3 Special information for cloning using restriction enzymes *Nde* I and *Not* I

- *Nde* I is sensitive to impurities in DNA preparations. To avoid cleavage at lower rates, make sure that your DNA preparations are highly pure (DNA purified by "quick-and-dirty" miniprep procedures is often *NOT* pure enough). If necessary, increase *Nde* I concentrations used for restriction digest.
- DNA digested with *Nde* I is difficult to ligate with T4 DNA ligase. The ligation efficiency can be increased by adding 15% polyethylenglycol (PEG).
- *Not* I inefficiently cuts supercoiled plasmids. Linearize the DNA with the other enzyme or use up to 5-fold more *Not* I for complete digestion.

4.1.4 Subcloning of PCR fragments using PCR cloning vectors

A disadvantage of direct cloning may be the inefficient cutting of restriction sites located at the very end of a fragment in some cases. As the restriction digest creates only a small difference in the fragment size, incomplete digestion will not be easily visible on agarose gels.

continued on next page

Subcloning in PCR cloning vectors may avoid this problem.

IF you want to...	THEN...
Subclone in blunt end cloning vectors	• Perform the PCR with thermostable *Tgo* DNA polymerase (with 3'-5'-Exonuclease activity) to create PCR fragments with blunt ends (the Expand High Fidelity PCR-System also creates a sufficient amount of blunt ended PCR fragments). • Then ligate into a blunt end cut cloning vector (e.g. using the PCR Cloning Kit). • Cut out the template gene from the subcloning vector and clone into the pIVEX vector cut with compatible restriction enzymes.
Subclone in T-overhang cloning vectors	• Perform the PCR with Expand High Fidelity PCR-System or *Taq* DNA Polymerase to create PCR fragments with single deoxyadenosine residue overhangs at the 3'ends. • Then ligate into a linearized cloning vector with a T-overhang and continue as described above.

Literature

For information on basic cloning techniques, please refer to the following general references:

1 Sambrook et al. (1989) "Molecular Cloning - A Laboratory Manual" Second Edition, Cold Spring Harbor Laboratory Press, New York.

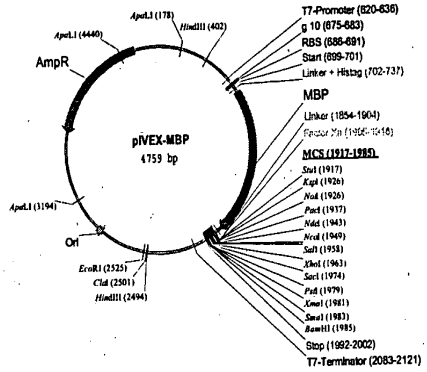
2 Ausubel, U. K. et al. (1993) "Current Protocols in Molecular Biology" John Wiley & Sons Inc., New York.

4.2 Trouble shooting guide

Observation	Potential Reason	Recommendation
No PCR product	Secondary structures of the primers	• Try to minimize secondary structure and dimer formation when designing primers. • Raise the primer concentration in the PCR reaction or use longer primers without G or C nucleotides at the 3'-end if a G+C content of 60% is not feasible.
	Inadequate annealing temperature	• Check whether the right annealing temperature was used for the PCR reaction (5 to 10°C lower than T_m). • Adapt the annealing temperature to the primer with the lowest melting temperature.
	Concentration of $MgCl_2$ too low	• Determine the optimal $MgCl_2$ concentration specifically for each template/primer pair by preparing a reaction series containing 0.5–4.5 mM $MgCl_2$. • Optimize the concentration of template DNA in the PCR reaction.
Nonspecific amplification	Low specificity of the primers	• Make sure that the primers specifically flank the 5'- and 3'- ends of your gene and are not complementary to other sequence regions of the template DNA. If necessary, increase primer length. • Use hot start techniques.
	Concentration of $MgCl_2$ too high	• Avoid excess of free magnesium leading to unspecific amplification. • Determine the optimal concentration by preparing a reaction series containing 0.5–4.5 mM $MgCl_2$. • Raise the annealing temperature if necessary.
No or only few colonies after transformation	Inappropriate selection medium	Make sure that your plates contain 50 µg/ml ampicillin or carbenicillin and no other antibiotics.
	Inactive competent cells	• Avoid frequent freezing and thawing of competent cells. • Perform a test transformation with 10 pg supercoiled control plasmid.
	Excess of ligation reaction during transformation	• Limit the volume of the ligation reaction to less than 20% of the whole transformation reaction volume to avoid inhibitory effects due to ligation buffers.
	Unsuccessful restriction digest of the PCR product	• Make sure that the right restriction buffer and reaction conditions were chosen. ***Note:*** *Sma* I is optimally active at 25°C. • For restriction digest with *Nde* I and *Not* I, see appendix (section 4.1.3). • Increase incubation time. • Subclone the PCR product into a PCR cloning vector if direct cloning after digestion of the PCR product is not successful (see section 4.1.5).
	Unsuccessful ligation	• Check activity of T4 DNA ligase by performing a control ligation reaction. • Use fresh ligase • Store the ligation buffer aliquoted at -20°C, as freezing and thawing results in degradation of ATP. • Vary the ratio of vector DNA to insert DNA: • Adjust the molar ratio of vector DNA to insert DNA to 1+3 (e.g. 50 ng linearized dephosphorylated vector and 50 ng insert (for a vector / insert size ratio of 3:1). • When vector and insert DNA differ in length, try other molar ratios (1+1, 1+2). • Use restriction enzymes providing sticky ends at both ends of the gene fragment to be cloned (e.g. use *Xma* I instead of *Sma* I). ***Note:*** For ligation of DNA fragments digested with *Nde* I, see appendix (section 4.1.3).
	Neither insert nor vector has 5'-phosphate residues necessary for ligation reaction	Make sure that either vector or insert is phoshorylated: • use a 5'-phosphorylated sense primer or • treat the insert with T4 polynucleotide kinase or • do not dephosphorylate the vector.
	Alkaline phosphatase not inactivated after vector dephosphorylation	Inactivate the alkaline phosphatase (please note: shrimp alkaline phosphatase can be inactivated simply by heat treatment whereas complete inactivation of calf intestine phosphatase requires additional treatments (e.g. phenolization).
High background of non-recombinants after transformation	Inappropriate medium	Make sure that your selection medium contains the correct, active antibiotic by performing a mock transformation reaction without DNA. No colonies should be obtained.
	Incomplete digestion of vector / insert	Purify the vector / insert after the first digestion step using the High Pure PCR Product Purification Kit. Perform the second digestion step of the vector / insert in the optimal buffer.
	Unsuccessful dephosphorylation of the vector	• Perform a religation control reaction without insert where only few colonies should be obtained. • Use fresh (shrimp) alkaline phosphatase. • Increase the incubation time.
	Excess of linearized, phosphorylated vector	• Depending on background strongly reduce the amount of linearized vector in the ligation reaction two- to fivefold. ***Note:*** If the vector/insert ratio is too high, religation is favored.

4.3 Vect r map

pIVEX-MBP




```
                                T7-Promoter
601  GATCTCGATC CCGCGAAATT AATACGACTC ACTATAGGGA GACCACAACG
     CTAGAGCTAG GGCGCTTTAA TTATGCTGAG TGATATCCCT CTGGTGTTGC

                                g10 e       RBS
651  GTTTCCCTCT AGAAATAATT TTGTTTAACT TTAAGAAGGA GATATACCAT
     CAAAGGGAGA TCTTTATTAA AACAAATTGA AATTCTTCCT CTATATGGTA
                                                      Me

      Linker    Histag                          MBP + Linker
701  GTCTGGTTCT CATCATCATC ATCATCATAG CAGCGGTATG CATAAAATCG
     CAGACCAAGA GTAGTAGTAG TAGTAGTATC GTCGCCATAC GTATTTTAGC
     tSerGlySer HisHisHisH isHisHisSe rSerGlyMet HisLysIleG

     [...]

                                NotI
        Factor Xa StuI     KspI       PacI      NdeI   NcoI
1901 CGGGATCGAA GGCAGGCCTG GCCGCGGCCG CTTAATTAAA CATATGACCA
     GCCCTAGCTT CCGTCCGGAC CGGCGCCGGC GAATTAATTT GTATACTGGT
     uGlyIleGlu GlyArgProG lyArgGlyAr gLeuIleLys HisMetThrM

                                XmaI
          SalI  XhoI  SacI  PstI  SmaI BamHI
1951 TGGCAAGTCG ACTCGAGCGA GCTCTGCAGC CCGGGATCCG GTAACTAACT
     ACCGTTCAGC TGAGCTCGCT CGAGACGTCG GGCCCTAGGC CATTGATTGA
     etAlaSerAr gLeuGluArg AlaLeuGlnP roGlyIleAr g***
```

4.4 Detection of expressed fusion proteins

The His-tagged proteins can be detected easily after SDS-PAGE and by Western blotting using an Anti-His_6 antibody. For methods in basic procedures refer to the literature (e.g. Ausubel et al., see chapter 4.1.4).
For Cat. No. of the products needed for detection, please refer to section 4.7.

Step	Action
1	Dilute the Western Blocking Reagent 1:10 in TBST (50 mM Tris/HCl, 150 mM NaCl, 0.1 % (v/v) Tween 20, pH 7.5) and incubate the blot in 20 ml of this blocking buffer for 90 min at room temperature (or at 4°C overnight).
2	Wash 3 × 5 min with TBST.
3	Dissolve Anti-His_6-Peroxidase at a concentration of 50 U/ml in water.
4	Incubate the blot in 50 ml blocking buffer with 12.5 µl of the Anti-His_6-Peroxidase solution (final concentration 12.5 mU/ml Anti-His_6 Peroxidase) for 60 min at room temperature with gentle agitation.
5	Wash 4 × 5 min with TBST.
6	Incubate the blot for 5 min in a quantity of Lumi-Light ^{Plus} substrate solution sufficient to cover the membrane (0.1 ml/cm^2).
7	Expose on Lumi-Imager F1 Work Station or X-ray film for 1 min. Adjust the exposure time between 10 s and 20 min according to the result of the first film.

Example:

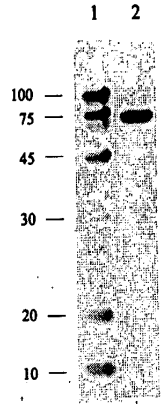


Fig. 2: : Expression of MBP-CAT (2) in RTS 100 HY: Western blot was incubated with Roche's Anti-His_6-POD conjugate as described. (1) = Multi-Tag-Marker

4.5 Note to the purchaser

The MBP expression component (derived from pMAL™ New England Biolabs) of Roche's Rapid Translation System (RTS) Reagents and Kits is licensed for use with Roche's Rapid Translation System only. For any commercial uses of pMAL™ expression, licensing information may be obtained from New England Biolabs, Legal Department, 32 Tozer Road, Beverly, MA 01915, USA.

When using the Ni-NTA technology for the purification of polyhistidine-tagged proteins in research applications, it is recommended to purchase the purification resin from Qiagen for which they hold exclusive licenses from F. Hoffmann-La Roche under European Patent 0253303, US Patent 4,877,830 and corresponding patent rights.

When using the Ni-NTA technology and the purificationresin from Qiagen for commercial purposes, a license is required in addition from F. Hoffmann-LaRoche under the above mentioned patents.

4.6 How to contact Roche Applied Science

Three ways to contact us

To contact Roche Applied Science for technical assistance, please choose one of the following:

IF you are using...	THEN...
the Internet	Access our web site at: http://www.proteinexpression.com or http://www.roche-applied-science.com
E-mail	Please refer to the address that corresponds to your particular location, printed on the last page of this instruction manual.
the telephone	Please refer to the telephone number that corresponds to your particular location, printed on the last page of this instruction manual.

4.7 Related products

Product	Pack size	Cat. No.
Rapid Translation System RTS pIVEX His-tag, 2nd Generation Vector Set	2 × 10 µg	3 269 019
Rapid Translation System RTS pIVEX His-tag Vector Set	5 × 10 µg	3 253 538
Rapid Translation System RTS pIVEX HA-tag Vector Set	2 × 10 µg	3 268 993
Rapid Translation System RTS 100 *E. coli* HY Kit	24 reactions (50 µl each) 96 reactions	3 186 148 3 186 156
Rapid Translation System RTS 500 Instrument	1 instrument	3 064 859
Rapid Translation System RTS 500 *E. coli* Circular Template Kit	5 reactions (1 ml each)	3 018 008
Rapid Translation System RTS 500 *E. coli* HY Kit	2 reactions (1 ml each) 5 reactions (1 ml each)	3 246 817 3 246 949
Rapid Translation System RTS GroE Supplement	5 × 125 µl	3 263 690
Tgo DNA Polymerase	50 reactions	3 186 172
Expand High Fidelity PCR System	100 units	1 732 641
PCR Cloning Kit (blunt end)	1 kit	1 939 645
Agarose MP	100 g	1 388 983
Agarose Gel DNA Extraction Kit	1 kit	1 696 505
High Pure PCR Product Purification Kit	1 kit	1 732 668
Geno Pure Plasmid Midi Kit	20 preparations	3 143 414
Geno Pure Plasmid Maxi Kit	10 preparations	3 143 422
Rapid DNA Ligation Kit	1 kit	1 635 379
Phosphatase, alkaline, shrimp	1000 units	1 758 250
Multi-Tag-Marker	250 µl	1 828 649
Anti-His_6-Peroxidase	50 units	1 965 085
Western Blocking Reagent	100 ml	1 921 673
Lumi-Light^{Plus} Western Blotting Substrate	100 ml	2 015 196
Restriction Enzymes (for a complete listing of all restriction enzymes and pack sizes, refer to the Roche Applied Science Catalog)		
Bam HI	1000 units	220 612
Bse AI	200 units	1 417 169
Bsp LU11I	200 units	1 693 743
Eco RV	2000 units	667 145
Nco I	200 units	835 315
Nde I	200 units	1 040 219
Not I	200 units	1 014 706
Pin AI (*Age* I)	200 units	1 464 841
Rca I (= *Bsp* HI)	200 units	1 467 123
Sca I	500 units	775 258
Sgr AI	200 units	1 277 014
Sma I	1000 units	220 566
Ssp I	200 units	972 967
Xba I	1000 units	674 257
Xma CI (=*Xma* I)	200 units	1 743 392

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